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Applicant(s)	: James Harrison Aylward	Docket	: 14923Z
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Commissioner of
Patent and Trademarks
Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R. §1.132

I, Dr. James Harrison Aylward, hereby declare as follows:

1. I am currently the Research Director of Peplin Operations Pty Ltd, a subsidiary of Peplin Biotech Ltd, Ground Floor, South Tower, 527 Gregory Terrace, Bowen Hills, Brisbane, QLD, 4006, Australia. My Curriculum Vitae is attached hereto as Exhibit **JHA-1**.

2. I have published extensively in the area of biochemistry. A list of my publications is included in my Curriculum Vitae (Exhibit **JHA-1**).

3. I am an inventor of subject matter contained and described in United States Patent Application Serial No. 09/888,178 filed on 21 June, 2001 (hereinafter referred to as the "APPLICATION"). The APPLICATION is directed *inter alia* to a method for treating cancer by administering to the subject in need thereof a therapeutically effective amount of an angeloyl-substituted ingenane obtainable from the sap of a

Euphorbia species and an active derivative of an angeloyl-substituted ingenane obtainable from the sap of a *Euphorbia* species.

4. In conjunction with my scientific collaborators, I conducted experiments isolating ingenanes from *Euphorbia* species using methodologies such as HPLC. The following experiments describe the isolation of 16 ingenane compounds from *Euphorbia paralias*.

In the following Examples, ^{1}H NMR and ^{13}C NMR data for compounds 1-7 are shown in Tables 1 and 2 respectively; ^{1}H NMR data for compounds 8-12 are shown in Table 3, ^{13}C NMR data for compounds 8, and 13-16 are shown in Table 4,; ^{1}H NMR data for compounds 13-16are shown in Table 5 and the structures of the compounds are shown in Table 6.

The isolated compounds 1-16 were tested for anticancer activity. All had activity at least greater than 100 bipolar units, as measured by reversion of malignant melanoma MM96L cells to a bipolar dendritic morphology, the assay as described in United States Patent Application Serial No. 09/888,178 filed on 21 June, 2000.

Isolation and Identification of Ingenanes from *Euphorbia paralias* plants

Example 1:

Euphorbia paralias plants collected form the coastline of Victoria, Australia were washed with water and the roots removed. The stems and leaves were cut into *ca* 1cm lengths and stood in water (2l per 500g of plant material) for at least 1 hour then filtered through glass fibre paper. The plant material was then stood in a further 1l of water for at least 1 hour then filtered through glass fibre paper. The combined filtrates from 20kg of stems and leaves of *Euphorbia paralias* treated in this manner were passed through a column of XAD-2 resin (1kg) at a rate of 10-20ml/hour. The XAD-2 resin was then washed with 40% methanol in water (10l) then 100% methanol (8l). The first 500ml of methanol was discarded and the remaining 7.5l combined and concentrated to a brown foam (13.4g).

Example 2:

Samples (2.0g) of *Euphorbia paralias* extract prepared as per Example 1 were taken up in methanol (5ml) and loaded onto a 3.4cm ID x 41cm column of Sephadex LH-20. This was eluted with 10% water in methanol at a drop rate of 1.4ml/min. 20ml eluate fractions were collected and analysed by HPLC on a 150mm x 4.6mm ID Alltima C18 5u column. These were combined and concentrated as follows, in order of elution, into:

Fractions containing polar material and no diterpenes

Fractions containing mainly polar material and some diterpenes

Fractions containing mainly segetanes, paralianes and jatrophanes with small amounts of ingenanes

Fractions containing mainly ingenanes

Fractions containing mainly polar material but some ingenanes

Fractions containing polar material and no diterpenes

Fractions containing diterpenes were dissolved in methanol (1ml/g) and loaded in 1g quantities onto a 2.6cm ID x 88cm column of Sephadex LH-20. This was eluted with 10% water in methanol at a drop rate of 0.4ml/min. 2ml eluate fractions were collected and analysed by HPLC. These were combined and concentrated as follows, in order of elution, into:

Fraction 1: containing polar material and no diterpenes

Fraction 2: containing polar material, segetanes, jatrophanes and paralianes

Fraction 3: containing segetanes, jatrophanes and paralianes

Fraction 4: containing mainly segetanes, jatrophanes, paralianes but some ingenanes

Fraction 5: containing segetanes, jatrophanes, paralianes and ingenanes but little 3-angeloyl-20-deoxyingenol

Fraction 6: containing segetanes, jatrophanes, paralianes and ingenanes, predominantly 3-angeloyl-20-deoxyingenol

Fraction 7: containing polar material and 3-angeloyl-20-deoxyingenol

Fraction 8: containing polar material and no ingenanes

Example 3:

Samples of fractions from Example 2 were subjected in *ca* 100mg quantities to HPLC on a 22mm ID x 250mm Alltima C18 5u column under the following conditions: 70% methanol isocratic for 30 mins. at 8ml/min., increasing linearly to 89.5% methanol at 9.3ml/min. over 200 mins., then again linearly to 100% methanol at 10ml/min. over 5 mins., and isocratic at 100% methanol for 25 mins.

Example 4:

Analytical HPLC analysis was conducted on a 4.6mm ID x 150mm Alltima C18 5u column, eluted at 1ml/min. with 75% methanol in water for 10 mins. followed by a linear gradient to 100% methanol over 15 mins. then 100% methanol for 7 mins. The eluate was monitored at 230 and 254nm.

Example 5:

Concentration of the eluate fractions containing the peak at 138 mins. from HPLC of fraction 5 from Example 2 according to Example 3 gave a colourless gum (25mg). This was taken up in *tert*-butyl methyl ether (MTB) and subjected to HPTLC on a Merck 10 x 20cm HPTLC plate coated with LiChrospher $Si60F_{254s}$ (eluent 50% MTB in 40-60° bp petroleum spirit). Concentration of the ether extract of the excised band with R_f 0.54 gave 20-hydroxy-3,17-bis(angeloyloxy)-4,5-dihydroxyingena-1,6-dien-9-one (compound 1) (4mg) as a colourless gum. HPLC r.t. 20.1 mins. (according to Example 4). HRMS m/z 528.2704, calcd for $C_{30}H_{40}O_8$ 528.2723. $APCIMS^+$ m/z 551 (6) $[M+Na]^+$, 529 (2) $[M+H]^+$, 511 (22) $[M-OH]^+$, 411 (28) [M-angelic acid, $-OH]^+$, 311 (100) [M-angelate, -angelic acid, $-H_2O]^+$, 293 (44) [M-angelate, -angelic acid, $-2H_2O]^+$. $APCIMS^-$ m/z 527 (7) $[M-H]^-$, 427 (65) [M-H, -angelic acid]$^-$, 410 (47) [M-angelic acid, $H_2O]^-$, 409 (47) [M-H, -angelic acid, $H_2O]^-$, 327 (57) [M-H, -2angelic acid]$^-$, 310 (96) [M-2angelic acid, $-H_2O]^-$, 309 (100) [M-H, -2angelic acid, $-H_2O]^-$, 297 (49).

Example 6:

Concentration of the eluate fractions containing the peak at 157 mins. from the HPLC of fraction 5 from Example 2 according to Example 3 gave a colourless gum (29mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20cm HPTLC plate (eluent 40% MTB in 40-60° bp petroleum spirit). Concentration of the ether extract of the excised band with R_f 0.91 gave 3,17-bis(angeloyloxy)-4,5-dihydroxyingena-1,6-dien-9-one (compound 2) (11mg) as a colourless gum. HPLC r.t. 23.2 mins. (according to Example 4). HRMS m/z 512.2772, calcd for $C_{30}H_{40}O_7$ 512.2774. APCIMS$^+$ m/z 535 (12) $[M+Na]^+$, 513 (7) $[M+H]^+$, 495 (11) $[M-OH]^+$, 395 (33) [M-angelate, $-H_2O]^+$, 313 (100) [M-angelic acid, -angelate]$^+$, 295 (85) [M-angelic acid, -angelate, $-H_2O]^+$. APCIMS$^-$ m/z 547 (100) [M ($C_{30}H_{40}O_7$)+Cl]$^-$, 511 (3) $[M-H]^-$, 311 (19) [M-H, -2angelic acid]$^-$.

Example 7:

Concentration of the eluate fractions containing the peak at 157 mins. from the HPLC of fraction 5 from Example 2 according to Example 3 gave a colourless gum (29mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20cm HPTLC plate (eluent 40% MTB in 40-60° bp petroleum spirit). Concentration of the ether extract of the excised band with R_f 0.75 gave 5,17-bis(angeloyloxy)-3,4-dihydroxyingena-1,6-dien-9-one (compound 3) (7mg) as a colourless gum. HPLC r.t. 16.3 mins. (according to Example 4). HRMS m/z 512.2780, calcd for $C_{30}H_{40}O_7$ 512.2774. APCIMS$^+$ m/z 535 (14) $[M+Na]^+$, 513 (1) $[M+H]^+$, 495 (7) $[M-OH]^+$, 413 (34) [M-angelate]$^+$, 393 (33) [M-angelate, $-H_2O]^+$, 313 (65) [M-angelic acid, -angelate]$^+$, 295 (100) [M-angelic acid, -angelate, $-H_2O]^+$. APCIMS$^-$ m/z 547 (100) [M ($C_{30}H_{40}O_7$)+Cl]$^-$, 511 (2) $[M-H]^-$.

Example 8:

Concentration of the eluate fractions containing the peak at 147 mins. from the HPLC of fraction 5 from Example 2 according to Example 3 gave a colourless gum (77mg). This was taken up in MTB and subjected to HPTLC on three Merck 10 x 20 HPTLC plates (eluent 40% MTB in 40-60° bp petroleum spirit). Concentration of the ether

extract of the excised band with R_f 0.57 gave 20-acetoxy-3,17-bis(angeloyloxy)-4,5-dihydroxyingena-1,6-dien-9-one (compound 4) (12mg) as a colourless gum. HPLC r.t. 22.0 mins. (according to Example 4). HRMS m/z 570.2828, calcd for $C_{32}H_{42}O_9$ 570.2829. APCIMS$^+$ m/z 593 (29) $[M+Na]^+$, 511 (30) $[M-OAc]^+$, 471 (26) [M-angelate]$^+$, 411 (18) [M-angelic acid, -OAc]$^+$, 393 (30) [M-angelic acid, -OAc, -H_2O]$^+$, 311 (100) [M-angelate, -angelic acid, -AcOH]$^+$, 293 (96) [M-angelate, -angelic acid, -AcOH, -H_2O]$^+$, 265 (33) [M-angelate, -angelic acid, -AcOH, -H_2O, -CO]$^+$. APCIMS$^-$ m/z 605 (35) $[M+Cl]^-$, 569 (35) $[M-H]^-$, 509 (85) [M-H, AcOH]$^-$, 452 (4) [M-angelic acid, -H_2O]$^-$, 427 (32) [M-H, -angelic acid, -CH_2CHO]$^-$, 410 (100) [M-angelic acid, -AcOH]$^-$, 409 (53) [M-H, -angelic acid, -AcOH]$^-$, 327 (33) [M-H, -2angelic acid, -CH_2CHO]$^-$, 310 (73) [M-2angelic acid, -AcOH]$^-$, 309 (38) [M-H, -2angelic acid, -AcOH] 292 (30) [M-2angelic acid, -AcOH, -H_2O]$^-$.

Example 9:

Concentration of the eluate fractions containing the peak at 147 mins. from the HPLC of fraction 5 from Example 2 according to Example 3 gave a colourless gum (77mg). This was taken up in MTB and subjected to HPTLC on three Merck 10 x 20 HPTLC plates (eluent 40% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.49 gave (compound 5) (13mg) as a colourless gum. HPLC r.t. 11.2 mins. (according to Example 4). HRMS m/z 570.2824, calcd for $C_{32}H_{42}O_9$ 570.2829. APCIMS$^+$ m/z 593 (9) $[M+Na]^+$, 571 (3) $[M+H]^+$, 511 (28) $[M-OAc]^+$, 411 (20) [M-angelic acid, -OAc]$^+$, 311 (100) [M-angelate, -angelic acid, -AcOH]$^+$, 293 (49) [M-angelate, -angelic acid, -AcOH, -H_2O]$^+$. APCIMS$^-$ m/z 569 (6) $[M-H]^-$, 427 (20) [M-H, -angelic acid, -CH_2CHO]$^-$, 410 (53) [M-angelic acid, -AcOH]$^-$, 409 (31) [M-H, -angelic acid, -AcOH]$^-$, 327 (10) [M-H, -2angelic acid, -CH_2CHO]$^-$, 310 (100) [M-2angelic acid, -AcOH]$^-$, 309 (43) [M-H, -2angelic acid, -AcOH].

Example 10:

Concentration of the eluate fractions containing the peak at 147 mins. from the HPLC of fraction 5 from Example 2 according to Example 3 gave a colourless gum (77mg).

This was taken up in MTB and subjected to HPTLC on three Merck 10 x 20 HPTLC plates (eluent 40% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.26 gave 17,20-bis(angeloyloxy)-3,4,5-trihydroxyingena-1,6-dien-9-one (compound 6) (0.4mg) as a colourless gum. HPLC r.t. 14.6 mins. (according to Example 4). HRMS m/z 528.2714, calcd for $C_{30}H_{40}O_8$ 528.2723. APCIMS$^+$ m/z 551 (15) $[M+Na]^+$, 511 (12) $[M-OH]^+$, 429 (23), 411 (20) [M-angelic acid, -OH]$^+$, 393 (20), 311 (100) [M-angelate, -angelic acid, -H_2O]$^+$, 293 (44) [M-angelate, -angelic acid, -$2H_2O$]$^+$. APCIMS$^-$ m/z 527 (7) $[M-H]^-$, 427 (55) [M-H, -angelic acid]$^-$, 410 (9) [M-angelic acid, -H_2O]$^-$, 327 (19) [M-H, -2angelic acid]$^-$, 310 (12) [M-2angelic acid, -H_2O]$^-$.

Example 11:

Concentration of the eluate fractions containing the peak at 147 mins. from the HPLC of fraction 5 from Example 2 according to Example 3 gave a colourless gum (77mg). This was taken up in MTB and subjected to HPTLC on three Merck 10 x 20 HPTLC plates (eluent 40% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.19 gave 5,17-bis(angeloyloxy)-3,4,20-trihydroxyingena-1,6-dien-9-one (compound 7) (2.1mg) as a colourless gum. HPLC r.t. 7.8 mins. (according to Example 4). HRMS m/z 528.2711, calcd for $C_{30}H_{40}O_8$ 528.2723. APCIMS$^+$ m/z 551 (12) $[M+Na]^+$, 511 (14) $[M-OH]^+$, 429 (46), 411 (25) [M-angelic acid, -OH]$^+$, 393 (18), 329 (26) [M-angelate, -angelic acid], 311 (100) [M-angelate, -angelic acid, -H_2O]$^+$, 293 (58) [M-angelate, -angelic acid, -$2H_2O$]$^+$. APCIMS$^-$ m/z 527 (7) $[M-H]^-$, 427 (65) [M-H, -angelic acid]$^-$, 410 (52) [M-angelic acid, -H_2O]$^-$, 409 (69) [M-H, -angelic acid, -H_2O]$^-$, 327 (30) [M-H, -2angelic acid]$^-$, 310 (40) [M-2angelic acid, -H_2O]$^-$, 309 (100) M-H, -2angelic acid, -H_2O]$^-$, 297 (15), 219 (46).

Example 12:

Concentration of the eluate fractions containing the peak at 208 mins. from the HPLC of fractions 5 and 6 from Example 2 according to Example 3 gave a colourless gum (17mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20

HPTLC plate (eluent 40% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.10 gave 2'*E*, 4'*E*, 6'*E*-3-angeloyloxy-17-deca-2',4',6'-trienoyloxy-4,5,20-trihydroxyingena-1,6-dien-9-one (compound 8) (2.4mg) as a colourless gum. HPLC r.t. 27.5 mins. (according to Example 4). HRMS m/z 594.3181, calcd for $C_{35}H_{46}O_8$ 594.3193. APCIMS$^+$ m/z 617 (30) $[M + Na]^+$, 577 (78) $[M\text{-}OH]^+$, 477 (25) [M-angelate, $-H_2O$], 329 (20) [M-angelate, -decatrienoic acid], 311 (100) [M-angelate, -decatrienoic acid, $-H_2O$], 293 (52) [M-angelate, -decatrienoic acid, $-2H_2O$], 149 (36) $[C_9H_{13}CO]^+$. APCIMS$^-$ m/z 629 (15) $[M+Cl]^-$, 575 (21) $[M\text{-}H, H_2O]^-$, 494 (100) [M-angelic acid]$^-$, 493 (87) [M-H, -angelic acid]$^-$, 476 (45) [M-angelic acid, $-H_2O]^-$, 475 (42) [M-H, -angelic acid, $-H_2O]^-$. This sample was a mixture of two isomers at the decatrienoyl moiety.

Example 13:

Concentration of the eluate fractions containing the peak at 211 mins. from the HPLC of fractions 5 and 6 from Example 2 according to Example 3 gave a colourless gum (10mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20 HPTLC plate (eluent 40% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.14 gave 2'*E*, 4'*E*, 6'*E*-20-angeloyloxy-17-deca-2',4',6'-trienoyloxy-3,4,5-trihydroxyingena-1,6-dien-9-one (compound 9) (0.7mg) as a colourless gum. HPLC r.t. 11.2 mins. (according to Example 4). HRMS m/z 594.3198, calcd for $C_{35}H_{46}O_8$ 594.3193. APCIMS$^+$ m/z 617 (22) $[M + Na]^+$, 577 (15) $[M\text{-}OH]^+$, 495 (83) [M-angelate], 329 (18) [M-angelate, -decatrienoic acid], 311 (75) [M-angelate, -decatrienoic acid, $-H_2O$], 293 (42) [M-angelate, -decatrienoic acid, $-2H_2O$], 149 (100) $[C_9H_{13}CO]^+$. APCIMS$^-$ m/z 629 (10) $[M+Cl]^-$, 593 (10) $[M\text{-}H]^-$, 494 (64) [M-angelic acid]$^-$, 493 (100) [M-H, -angelic acid]$^-$.

Example 14:

Concentration of the eluate fractions containing the peak at 208 mins. from the HPLC of fractions 5 and 6 from Example 2 according to Example 3 gave a colourless gum (17mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20

HPTLC plate (eluent 40% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.12 gave 2'*E*, 4'*E*, 6'*E*-5-angeloyloxy-17-deca-2',4',6'-trienoyloxy-3,4,20-trihydroxyingena-1,6-dien-9-one (compound 10) (0.1mg) as a colourless gum. HPLC r.t. 27.5 mins. (according to Example 4). HRMS m/z 594.3209, calcd for $C_{35}H_{46}O_8$ 594.3193. APCIMS$^+$ m/z 617 (31) $[M + Na]^+$, 577 (62) $[M\text{-}OH]^+$, 495 (71) [M-angelate], 329 (23) [M-angelate, -decatrienoic acid], 311 (100) [M-angelate, -decatrienoic acid, $-H_2O$], 293 (68) [M-angelate, -decatrienoic acid, $-2H_2O$], 149 (93) $[C_9H_{13}CO]^+$. APCIMS$^-$ m/z 629 (7) $[M+Cl]^-$, 593 (6) $[M-H]^-$, 494 (45) [M-angelic acid]$^-$, 493 (46) [M-H, -angelic acid]$^-$, 475 (30) [M-H, -angelic acid, $-H_2O$]$^-$.

Example 15:

Concentration of the eluate fractions containing the peak at 224 mins. from the HPLC of fractions 5 and 6 from Example 2 according to Example 3 gave a colourless gum (15mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20 HPTLC plate (eluent 40% MTB in 40-60° bp petroleum spirit). Concentration of the ether extract of the excised band with R_f 0.73 gave 2'*E*, 4'*E*, 6'*E*-3-angeloyloxy-17-deca-2',4',6'-trienoyloxy-4,5-dihydroxyingena-1,6-dien-9-one (compound 11) (0.8mg) as a colourless gum. HPLC r.t. 28.7 mins. (according to Example 4). HRMS m/z 578.3228, calcd for $C_{35}H_{46}O_7$ 578.3244. APCIMS$^+$ m/z 601 (7) $[M+Na]^+$, 561 (6) $[M\text{-}OH]^+$, 461 (7) [M-angelate, $-H_2O$], 313 (38) [M-angelate, -decatrienoic acid], 295 (34) [M-angelate, -decatrienoic acid, $-H_2O$], 149 (55) $[C_9H_{13}CO]^+$, 59 (100). This sample was a mixture of isomers at the decatrienoyl moiety. This compound (1.1mg) can also be obtained from the eluate fractions containing the peak at 220 mins. from the HPLC of fractions 5 and 6 from Example 2 according to Example 3.

Example 16:

Concentration of the eluate fractions containing the peak at 224 minutes from the HPLC of fractions 5 and 6 from example 2 according to example 3 gave a colourless gum (15mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20 HPTLC plate (eluent 40% MTB in 40-60° bp petroleum spirit). Concentration of the

ether extract of the excised band with R_f 0.53 gave 2'*E*, *4'E*, 6'*E*-5-angeloyloxy-17-deca-2',4',6'-trienoyloxy-3,4-dihydroxyingena-1,6-dien-9-one (compound 12) (0.4mg) as a colourless gum. APCIMS$^+$ m/z 601 (4) $[M(C_{35}H_{46}O_7)+Na]^+$, 313 (39) [M-angelate, -decatrienoic acid], 295 (26) [M-angelate, -decatrienoic acid, -H_2O], 149 (65) $[C_9H_{13}CO]^+$. This sample was a mixture of isomers at the decatrienoyl moiety. A partial ^{1}H NMR of one of these is given in Table 3.

Example 17:

Fraction 4 from example 2 was subjected in *ca* 100mg quantities to HPLC on a 22mm ID x 250mm Alltima C18 5u column according to the following conditions: 70% methanol isocratic for 2 min increasing from 0-9ml/min, then increasing linearly to 85% methanol at 9ml/min over 108min, then again linearly to 100% methanol at 10ml/min over 5 minutes, and isocratic at 100% methanol for 20 minutes. Concentration of the eluate fractions containing the peak at 87 minutes gave a colourless gum (5mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20 HPTLC plate (eluent 20% MTB in 40-60° bp petroleum spirit). Concentration of the ether extract of the excised band with R_f 0.35 gave 5,20-bis(acetoxy)-3,17-bis(angeloyloxy)-4-hydroxyingena-1,6-dien-9-one (compound 13) (3.7mg) as a colourless gum. HPLC r.t. 23.8min (according to example 4).HPLC r.t. 23.8min (according to example 4). HRMS m/z 612.2917, calcd for $C_{34}H_{44}O_{10}$ 612.2935. APCIMS$^+$ m/z 635 (45) $[M+Na]^+$, 630 (100) $[M+NH_4]^+$, 613 (17) $[M+H]^+$, 553 (43) $[M-OAc]^+$, 513 (26) [M-angelate]$^+$, 453 [M-OAc, -angelic acid]$^+$, 393 (35) [M-OAc, -AcOH, -angelic acid]$^+$, 353 (37) [M-OAc, -2angelic acid]$^+$, 311 (20), 293 (48) [M-OAc, -AcOH, -2angelic acid]$^+$, 265 (12) [M-OAc, -AcOH, -2angelic acid, -CO]$^+$.

Example 18:

Concentration of the eluate fractions containing the peak at 147 minutes from the HPLC of fraction 5 from example 2 according to example 3 gave a colourless gum (77mg). This was taken up in MTB and subjected to HPTLC on three Merck 10 x 20 HPTLC plates (eluent 40% MTB in 40-60° bp petroleum spirit, 3 sweeps).

Concentration of the ether extract of the excised band with R_f 0.28 gave 5-acetoxy-3,17-bis(angeloyloxy)-4,20-dihydroxyingena-1,6-dien-9-one (compound 14) (1.5mg) as a colourless gum. HPLC r.t. 21.4min (according to example 4). HRMS m/z 570.2826, calcd for $C_{32}H_{42}O_9$ 570.2829. APCIMS$^+$ m/z 593 (10) $[M+Na]^+$, 571 (3) $[M+H]^+$, 511 (26) $[M-OAc]^+$, 411 (21) [M-angelic acid, -OAc]$^+$, 311 (100) [M-angelate, -angelic acid, -AcOH]$^+$, 293 (65) [M-angelate, -angelic acid, -AcOH, -H_2O]$^+$. APCIMS$^-$ m/z 569 (5) $[M-H]^-$, 427 (100) [M-H, -angelic acid, -CH_2CHO]$^-$, 410 (48) [M-angelic acid, -AcOH]$^-$, 409 (45) [M-H, -angelic acid, -AcOH]$^-$, 327 (45) [M-H, -2angelic acid, -CH_2CHO]$^-$, 310 (59) [M-2angelic acid, -AcOH]$^-$, 309 (65) [M-H, -2angelic acid, -AcOH].

Example 19:

Concentration of the eluate fractions containing the peak at 130 minutes from the HPLC of fraction 6 from example 2 according to example 3 gave a colourless gum (35mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20cm HPTLC plate (eluent 20% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.22 gave 5-angeloyloxy-3,4-dihydroxyingena-1,6-dien-9-one (compound 15) (8mg) as a colourless gum. HPLC r.t. 11.2min (according to example 4). HRMS m/z 414.2408, calcd for $C_{25}H_{34}O_5$ 414.2406. APCIMS$^+$ m/z 437 (15) $[M+Na]^+$, 415 (4) $[M+H]^+$, 397 (10) [M-OH]$^+$, 315 (41) [M-angelate]$^+$, 297 (100) [M-angelate, -H_2O]$^+$, 269 (38) [M-angelate, -H_2O, -CO]$^+$. APCIMS$^-$ m/z 827 (100) $[2M-H\}^-$, 449 (60) $[M+Cl]^-$, 413 (3) $[M-H]^-$.

Example 20:

Concentration of the eluate fractions containing the peak at 130 minutes from the HPLC of fraction 6 from example 2 according to example 3 gave a colourless gum (35mg). This was taken up in MTB and subjected to HPTLC on a Merck 10 x 20cm HPTLC plate (eluent 20% MTB in 40-60° bp petroleum spirit, 3 sweeps). Concentration of the ether extract of the excised band with R_f 0.33 gave 3-angeloyloxy-4,5-dihydroxyingena-1,6-dien-9-one (compound 16) (9mg) as a

colourless gum. HPLC r.t. 19.2min (according to example 4). HRMS m/z 414.2410, calcd for $C_{25}H_{34}O_5$ 414.2406. APCIMS$^+$ m/z 437 (7) $[M+Na]^+$, 415 (10) $[M+H]^+$, 397 (8) $[M-OH]^+$, 315 (100) [M-angelate]$^+$, 297 (96) [M-angelate, $-H_2O]^+$, 269 (20) [M-angelate, $-H_2O$, -CO]$^+$. APCIMS$^-$ m/z 449 (100) $[M+Cl]^-$, 413 (1) $[M-H]^-$.

Table 1. ^{1}H NMR data (CD_2Cl_2, 500MHz) for compounds 1-7

	δ (ppm)						
H	1	2	3	4	5	6	7
1	6.00 bs	6.02q	5.93 q	6.01 q	5.92 q	5.88 bd	5.89 q
3	5.58 s	5.50 bs	3.72 d	5.57 bs	3.77 d	4.41 d	3.77 d
5	4.02 bd	3.67 bd	5.22 bs	3.87 bd	5.38 bs	3.66 bd	5.37 bs
7	6.00 bd	5.71 ddq	5.83 ddq	6.06 bd	6.21 bd	6.08 bd	6.16 bd
8	4.26 bdd	4.14 bdd	4.34 bdd	4.21 bdd	4.42 bdd	4.23	4.42 bdd
11	2.52 ddq	2.45 ddq	2.38 ddq	2.49 ddq	2.44 ddq	2.32	2.45 ddq
12	2.34 ddd	2.33 ddd	2.36 ddd	2.32 ddd	2.33 ddd	2.36 ddd	2.34 ddd
12'	1.85 ddd	1.84 ddd	1.85 ddd	1.86 ddd	1.85 ddd	1.85 ddd	1.86 ddd
13	0.94 ddd	0.91 ddd	0.94 ddd	0.95 ddd	0.96 ddd	0.95 ddd	0.96 ddd
14	1.11 dd	1.08 dd	1.10 dd	1.13 dd	1.18 dd	1.13 dd	1.17 dd
16	1.15 s	1.14 s	1.15 s	1.15 s	1.16 s	1.15	1.15
17	4.35 d	4.32 d	4.33 d	4.31 d	4.30 s	4.31	4.33
17'	4.18 d	4.18 d	4.27 d	4.19 d		4.26	4.30
18	0.96 d	0.96 d	0.96 d	0.97 d	0.97 d	0.96	0.98 d
19	1.79 d	1.79 d	1.80 d	1.80 d	1.81 d	15.1 bs	1.81 d
20	4.16 bdd	1.76 bs	1.55 bs	4.73 bd	4.51 bd	4.74	3.90 bd
20'	4.09 bdd			4.44 bd	4.25 d	4.57	3.85 bd
3-OAng 2'-Me	1.90dq	1.91 dq		1.91 dq			
3-OAng 3'	6.16qq	6.17 qq		6.17 qq			
3-OAng 4'	1.99dq	1.99 dq		1.99 dq			
5-OAng 2'-Me			1.94 dq		1.91 dq		1.94 dq
5-OAng 3'			6.17qq		6.18 qq		6.20 qq
5-OAng 4'			1.99dq		1.99 dq		2.00 dq
17-OAng 2'-Me	1.88dq	1.88 dq	1.89 dq	1.88 dq	1.89 dq	1.88 dq	1.88 dq
17-OAng 3'	6.08qq	6.07 qq	6.09qq	6.08 qq	6.09 qq	6.09 qq	6.09 qq
17-OAng 4'	1.97dq	1.97 dq	1.98dq	1.96 dq	1.98 dq	1.98 dq	1.98 dq
20-OAng 2'-Me						1.86 dq	
20-OAng 3'						6.05 qq	
20-OAng 4'						1.92 dq	
20-OAc				2.04			
3-OH			2.44 d		2.53 d	3.02 bd	2.42 d
4-OH	3.58	3.51 bs	3.92 bs	3.52 s	3.97	#	3.88
5-OH	4.13 d	3.12 bd		3.43 d		3.10 d	
20-OH	2.20 t						1.91 m
	J (Hz)						
J 1,19	1	1.4	1.4	1.4	1	1.4	1
J3, 3-OH			6		6	6	6
J 5,5-OH	5	7		7		11	
J 7,8	4	5	4	5	4	5	5
J 8,14	12	12	12	12	12	12	12
J 11,12	3	3	3	3	3	3	3
J 11,12'	5	5	4	5	5	6	6
J 11,18	7	7	7	7	7	7	7
J 12,12'	16	16	16	16	16	16	16
J 12,13	9	9	9	9	9	9	9
J 12',13	6	6	6	6	#	#	6
J 13,14	8	8	8	8	8	8	8
J 17,17'	12	12	12	12		12	12
J 20,20'	12			12	12	13	13
J 20,20-OH J 20',20-OH	6						6
OAng J2'-Me,3'	1.5	1.5	1.4	1.4	1.4	1	1.4
OAng J2'-Me,4'	1.5	1.5	1.4	1.4	1.4	1	1.4
OAng J3',4'	7	7	7	7	7	7	7

unable to determine value

Table 2. ^{13}C NMR data (CD_2Cl_2, 125MHz) for compounds 1-7

	δ (ppm)						
C	1	2	3	4	5	6	7
1	132.2	132.6	130.2	132.2	129.7	129.4	129.4
2	136.9	136.5	140.0	136.9	140.3	139.3	140.1
3	82.7	83.2	80.7	82.8	80.0	80.4	80.2
4	85.4	85.5	85.6	85.4	85.7	84.4	85.7
5	76.9	77.7	76.9	74.8	75.0	73.6	74.8
6	140.6	138.4	135.8	137.1	134.7	137.7	139.3
7	127.3	123.6	125.3	128.5	130.8	126.5	127.8
8	43.6	43.5	44.1	43.7	44.4	43.7	44.0
9	206.0	206.1	206.5	205.7	206.2	205.2	206.0
10	72.5	72.5	73.4	72.6	73.8	72.6	73.7
11	39.0	39.3	39.9	39.1	39.6	39.8	39.6
12	31.3	313	31.3	31.3	31.5	30.7	31.6
13	24.7	24.5	24.8	24.7	24.7	24.0	24.8
14	24.0	24.3	24.4	24.0	24.1	23.5	24.2
15	28.2	28.1	28.2	28.1	28.1	27.6	28.1
16	24.8	24.8	24.3	24.8	24.7	15.1	24.8
17	65.6	65.7	65.7	65.5	65.6	65.2	65.8
18	17.1	17.0	17.3	17.2	17.7	16.7	17.7
19	15.9	16.1	15.7	15.9	15.6	15.1	15.5
20	67.4	22.2	21.8	66.9	66.8	65.9	65.3
3-OAng 1'	168.8	168.7		168.6			
3-OAng 2'	127.8	127.7		127.7			
3-OAng 2'-Me	21.0	21.0		21.0			
3-OAng 3'	140.0	140.3		140.4			
3-OAng 4'	16.23	16.3		16.3			
5-OAng 1'			167.6		167.3		168.2
5-OAng 2'			127.6		127.4		127.4
5-OAng 2'-Me			21.0		21.0		20.9
5-OAng 3'			140.4		141.1		141.2
5-OAng 4'			16.3		16.3		16.2
17-OAng 1'	168.6	168.6	168.7	168.5	168.6	168.2	168.6
17-OAng 2'	128.5	128.6	128.5	128.4	128.4	127.9	128.4
17-OAng 2'-Me	21.0	21.0	21.0	21.0	20.9	20.5	21.0
17-OAng 3'	138.1	138.0	138.0	138.1	138.2	137.8	138.1
17-OAng 4'	16.15	15.9	16.1	16.1	16.2	15.7	16.4
20-OAc 1'				170.9			
20-OAc 2'				21.3			
20-OAng 1'						167.6	
20-OAng 2'						127.9	
20-OAng 2'-Me						20.4	
20-OAng 3'						137.8	
20-OAng 4'						15.5	

Table 3. ^{1}H NMR data (CD_2Cl_2, 500MHz) for compounds 8-12

	δ (ppm)						
H	8 (isomer 1)	8 (isomer 2)	9	10	11 (isomer 1)*	11(isomer 2)*	12*
1	6.00	6.00	5.89 bs	5.90 bs	6.02 bs	6.02 bs	5.94 bs
3	5.59	5.59	3.87 bs	3.76 d	#	#	#
5	4.01	4.01	3.66 bd	#	3.66 bd	3.66 bd	#
7	6.01	6.01	6.07 bd	6.17 bd	5.72 bm	5.72 bm	#
8	4.25	4.25	4.23 bdd	4.40 bdd	4.13 bd	4.13 bd	#
11	2.52 ddq	2.52 ddq	2.45 ddq	2.44 ddq	2.43 ddq	2.43 ddq	#
12	2.33 ddd	2.33 ddd	2.33 ddd	2.33 ddd	2.32 ddd	2.32 ddd	#
12'	1.86 ddd	1.86 ddd	1.85 ddd	1.86 ddd	1.85 ddd	1.85 ddd	#
13	0.94 ddd	0.94 ddd	0.94 ddd	0.96 ddd	0.91 ddd	0.91 ddd	#
14	1.10 dd	1.10 dd	1.10 dd	1.15 dd	1.06 dd	1.06 dd	#
16	1.16 s	1.16 s	1.15 s	1.15 s	1.14 s	1.14 s	#
17	4.35	4.35	4.29 d	4.32 d	4.31 d	4.31 d	4.32
17'	4.16	4.16	4.23 d	4.26 d	4.19 d	4.19 d	4.26
18	0.96	0.96	0.96 d	0.98 d	0.96 d	0.96 d	0.95
19	1.79	1.79	1.83 bs	1.81 bs	1.78 bs	1.78 bs	1.80 bs
20	4.14	4.14	4.74 d	3.89 bm	1.76 bs	1.76 bs	#
20'	4.08	4.08	4.54 d	3.86 bm			
3-OAng 2'-Me	1.90 dq	1.90 dq			1.91 bs	1.91 bs	
3-OAng 3'	6.16 qq	6.16 qq			6.17 bq	6.17 bq	
3-OAng 4'	1.99 dq	1.99 dq			1.99 bd	1.99 bd	
5-OAng 2'-Me				1.94 dq			1.94 bs
5-OAng 3'				6.21 qq			6.18 bq
5-OAng 4'				2.00 dq			1.99 bd
20-OAng 2'-Me			1.85 dq				
20-OAng 3'			6.05 qq				
20-OAng 4'			1.91 dq				
decatrienoyl-2'	5.88	5.86	5.84 d	5.85 d	5.85	5.88	#
decatrienoyl-3'	7.74	7.27	7.27 dd	7.29 dd	7.27	7.74	7.29
decatrienoyl-4'	6.00	6.24	6.24 dd	6.27 dd	6.25	6.00	6.25
decatrienoyl-5'	6.31	6.55	6.56 dd	6.57 dd	6.57	6.31	#
decatrienoyl-6'	6.65	6.17	6.16 dd	#	6.15	6.65	#
decatrienoyl-7'	5.95	5.96	5.97 dt	#	5.98	5.94	5.98
decatrienoyl-8'	2.14	2.14	2.14 dt	2.14	2.11	2.16	#
decatrienoyl-9'	1.44	1.44	1.43 tq	1.43	1.43	1.45	#
decatrienoyl-10'	0.91	0.91	0.91 t	0.92	0.92	0.92	#
3-OH			#	2.38			#
4-OH	#	#	#	#	#	#	#
5-OH	4.13	4.13	2.95		3.05 d		
20-OH				1.88			
	J (Hz)						
J 1,19	#	#	#	#	#	#	#
J3, 3-OH			#	6			#
J 5,5-OH	5	5	10		8	8	
J 7,8	#	#	6	#	#	#	#
J 8,14	#	#	13	10	#	#	#
J 11,12	#	#	4	#	#	#	#
J 11,12'	#	#	#	#	#	#	#
J 11,18	7	7	7	7	#	#	#
J 12,12'	16	16	16	16	#	#	#

J 12,13	#	#	9	9	#	#	#
J 12',13	#	#	#	#	#	#	#
J 13,14	#	#	#	#	#	#	#
J 17,17'	12	12	12	#	12	12	#
J 20,20'	13	13	13	#			
J 20,20-OH, J20',20-OH	#	#		#			
OAng J2'-Me,3'	#	#	#	#	#	#	#
OAng J2'-Me,4'	#	#	#	#	#	#	#
OAng J3',4'	7	7	7	7	7	7	7
decatrienoyl J 2'3'	15	16	14	15	14	14	#
decatrienoyl J 3',4'	11	10	13	11	12	12	#
decatrienoyl J 4',5'	11	13	13	13	14	11	#
decatrienoyl J 5',6'	11	11	10	11	11	11	#
decatrienoyl J 6',7'	16	13	14	#	14	14	#
decatrienoyl J 7',8'	8	8	7	#	7	7	#
decatrienoyl J 8',9'	7	7	6	#	#	#	#
decatrienoyl J 9',10'	7	7	7	7	7	7	#

*incomplete spectrum, most J values not determinable
unable to determine value

Table 4. ^{13}C NMR data (CD_2Cl_2, 125MHz) for compounds 8-16 (I8)

	δ (ppm)				
C	8*	13	14*	15(Pe3)	16(I8)
1	132.2	132.1	132.2	127.2	133.0
2	136.3	136.6	136.4	139.7	136.1
3	82.5	82.0	82.2	80.2	83.4
4	#	86.3	86.2	85.1	85.5
5	76.8	75.1	75.4	76.5	77.8
6	#	134.3	140.4	135.0	138.0
7	127.1	131.1	128.0	125.6	124.4
8	#	43.7	43.5	44.0	43.9
9	#	204.8	#	207.0	206.7
10	71.8	72.5	72.5	72.9	72.4
11	38.5	39.1	39.2	39.5	39.4
12	31.0	31.3	31.3	31.0	31.5
13	#	23.8	24.5	23.1	23.5
14	24.1	24.5	23.9	23.3	23.9
15	27.7	28.3	28.3	24.0	24.5
16	24.0	24.7	23.9	28.2	28.8
17	65.2	65.6	65.7	15.3	15.6
18	16.5	16.9	16.8	17.2	16.2
19	15.2	15.7	15.6	15.2	15.7
20	67.0	66.1	65.0	21.4	22.2
3-OAng 1'	168.1	169.3	169.3		168.8
3-OAng 2'	127.1	127.9	128.5		127.7
3-OAng 2'-Me	20.3	21.0	21.0		21.0
3-OAng 3'	139.3	139.7	139.7		140.3
3-OAng 4'	15.4	16.1	16.2		15.9
5-OAng 1'				167.3	
5-OAng 2'				129.9	
5-OAng 2'-Me				20.4	
5-OAng 3'				139.2	
5-OAng 4'				15.7	
17-OAng 1'	168.6	168.6			
17-OAng 2'	128.5	128.5			
17-OAng 2'-Me	21.0	21.0			
17-OAng 3'	138.1	138.1			
17-OAng 4'	16.1	16.2			
5-OAc 1'	171.3	172.1			
5-OAc 2'	21.2	21.2			
20-OAc 1'	170.9				
20-OAc 2'	21.2				
decatrienyl-1'	#				
decatrienyl-2'	#				
decatrienyl-3'	#				
decatrienyl-4'	#				
decatrienyl-5'	#				
decatrienyl-6'	#				
decatrienyl-7'	#				
decatrienyl-8'	#				
decatrienyl-9'	#				
decatrienyl-10'	#				

*incomplete spectrum

unable to determine value

Table 5. ^{1}H NMR data (CD_2Cl_2, 500MHz) for compounds 13-16 (I8)

	δ (ppm)			
H	13	14	15 (Pe3)	16 (I8)
1	6.05 bs	6.05 bs	5.93 q	6.03 q
3	5.05 s	5.02 s	3.73 bd	5.48 s
5	5.38 bs	5.38 bs	5.21 bs	3.68 bs
7	6.21 bd	6.14 bd	5.84 dq	5.72 dq
8	4.39 bdd	4.38 bdd	4.19 ddt	4.01 bd
11	2.55 ddq	2.54 ddq	2.37 ddq	2.44 ddq
12	2.34 ddd	2.36 ddd	2.29 ddd	2.24 ddd
12'	1.85 ddd	1.84 ddd	1.75 ddd	1.74 ddd
13	0.96 ddd	0.95 ddd	0.69 ddd	0.66 ddd
14	1.14 dd	1.13 dd	0.88 dd	0.85 dd
16	1.15 s	1.15 s	1.05 s	1.04 s
17	4.27 d	4.31 d	1.12 s	1.07 s
17'	4.18 d	4.19 d		
18	0.98 d	0.98 d	0.95 d	0.95 d
19	1.76 d	1.75 d	1.80 d	1.79 d
20	4.55 d	3.85 bd	1.56 bs	1.77 bs
20'	4.16 d			
3-OAng 2'-Me	1.88 dq	1.87 dq		1.91 dq
3-OAng 3'	6.13 qq	6.15 qq		6.18 qq
3-OAng 4'	1.97 dq	1.97 dq		1.99 dq
5-OAng 2'-Me			1.93 dq	
5-OAng 3'			6.17 qq	
5-OAng 4'			1.99 dq	
17-OAng 2'-Me	1.88 dq	1.88 dq		
17-OAng 3'	6.08 qq	6.07 qq		
17-OAng 4'	1.97 dq	1.97 dq		
20-OAng 2'-Me				
20-OAng 3'				
20-OAng 4'				
5-OAc	2.23 s	2.28		
20-OAc	1.98 s			
3-OH			2.40 bd	
4-OH	3.40 bs	3.41 bs	3.91 bs	3.45 s
5-OH				3.14 bs
20-OH		1.78 m		
J 1,19	1	1	1.4	1.4
J3, 3-OH			6	
J 5,5-OH				
J 7,8	4	5	4	5
J 7,20			1.4	1.4
J 8,14	12	12	12	11
J 8,20			2	1.4
J 11,12	3	3	3	3
J 11,12'	6	7	5	5
J 11,18	7	7	7	7
J 12,12'	16	16	16	16
J 12,13	10	10	9	9
J 12',13	4	5	6	6
J 13,14	8	8	8	8
J 17,17'	11	11		
J 20,20'	12			
J 20,20-OH, J20',20-OH		#		
OAng J2'-Me,3'	1.4	1.4	1.4	1.4
OAng J2'-Me,4'	1.4	1.4	1.4	1.4
OAng J3',4'	7	7	7	7

Table 6: Examples of novel and known (previously described structures but anticancer activity previously not known) angeloyl substituted ingenanes from *Euphorbia paralias*, not *peplus*, *hirta* or *drummondii*.

Structures of Compounds 1-16

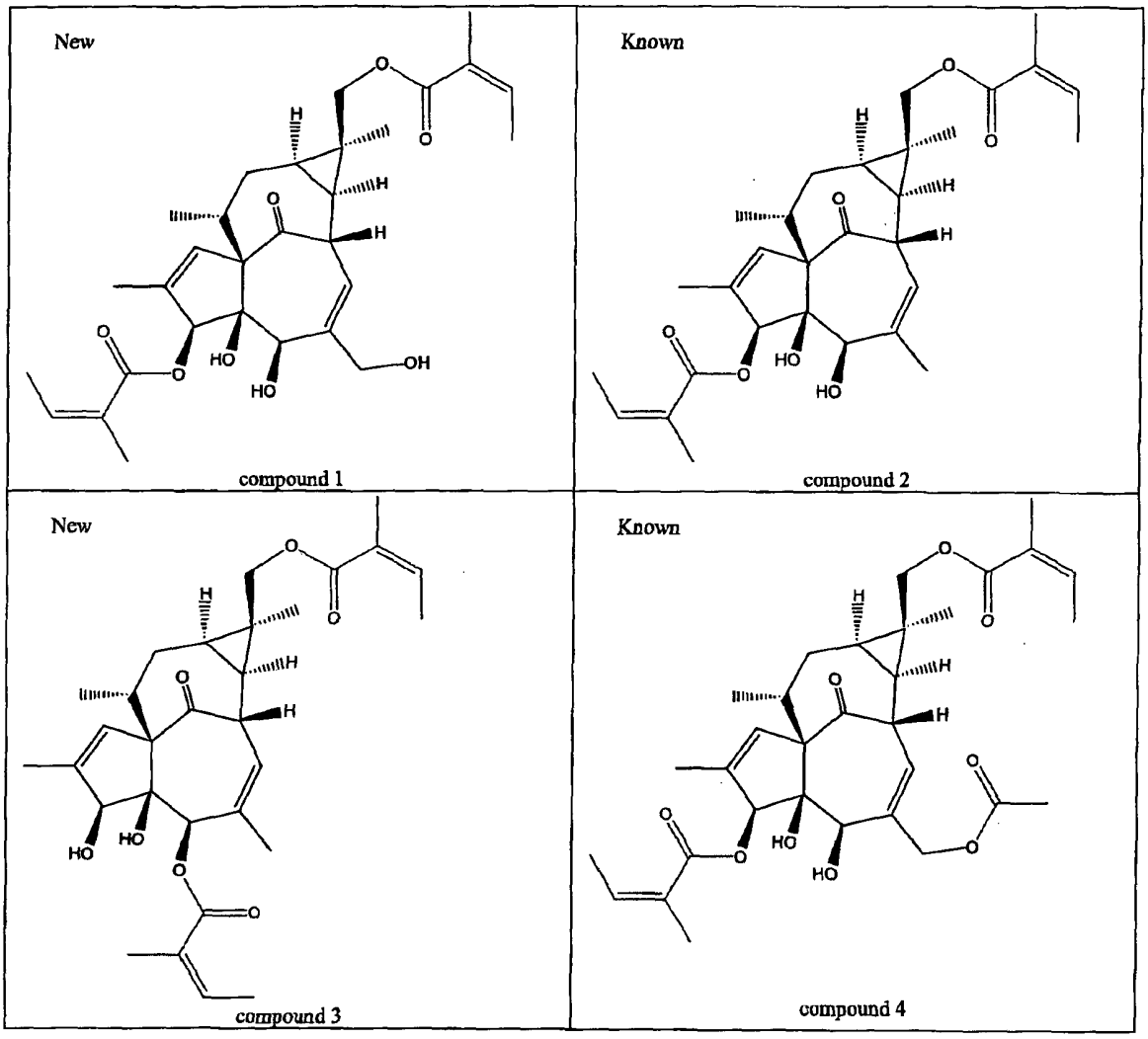


Table 6: Structures of Compounds 1-16 (c/fwd)

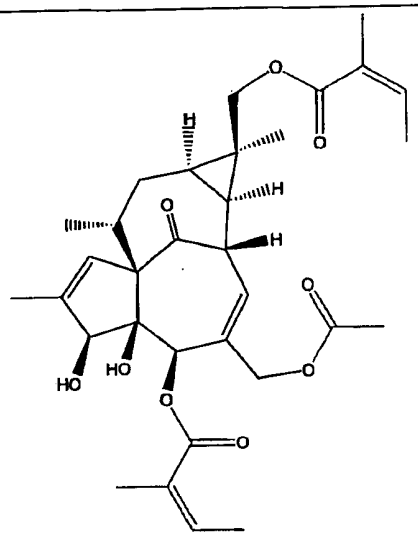
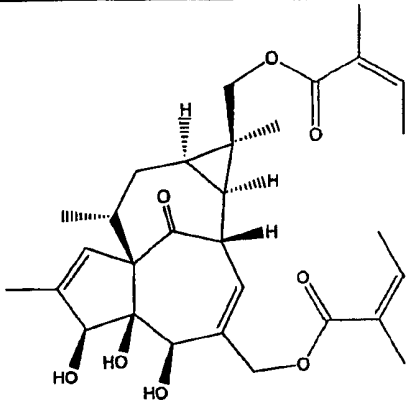
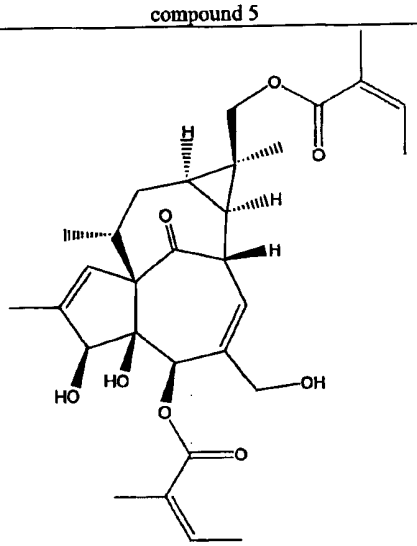
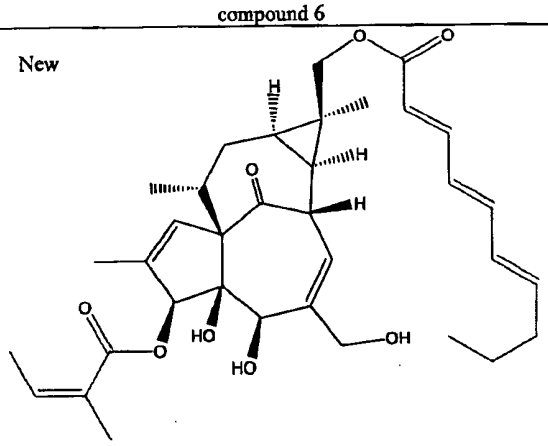
New  compound 5	New  compound 6
New  compound 7	New  compound 8

Table 6: Structures of Compounds 1-16 (c/fwd)

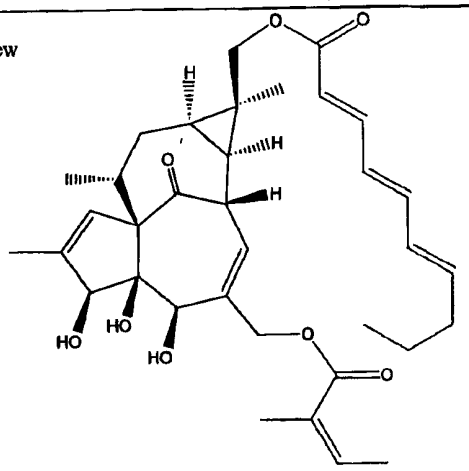
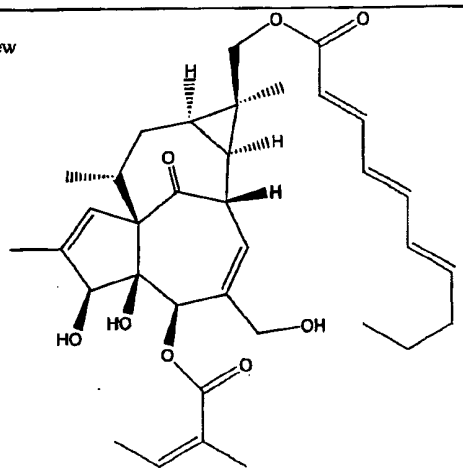
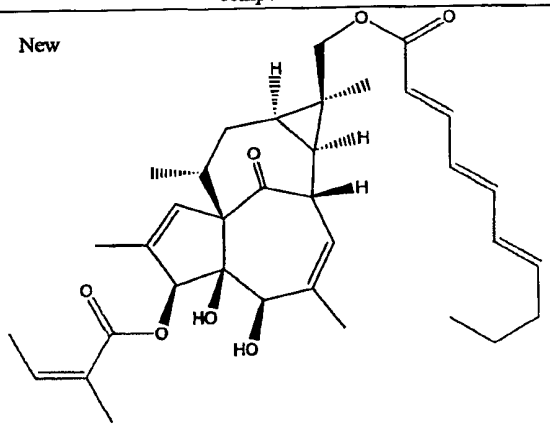
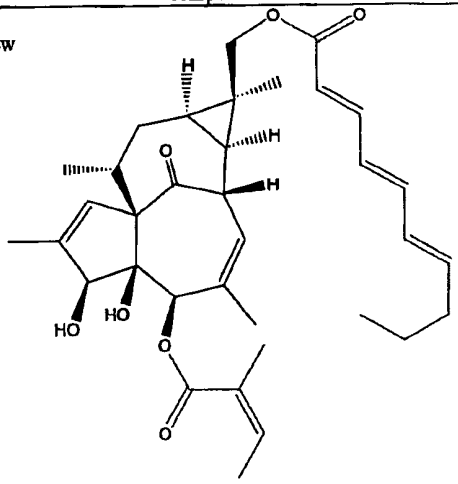
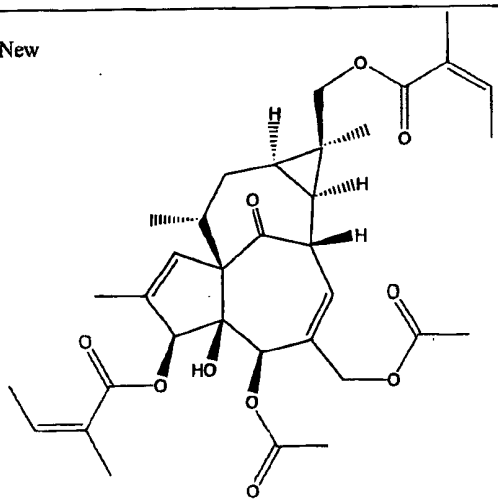
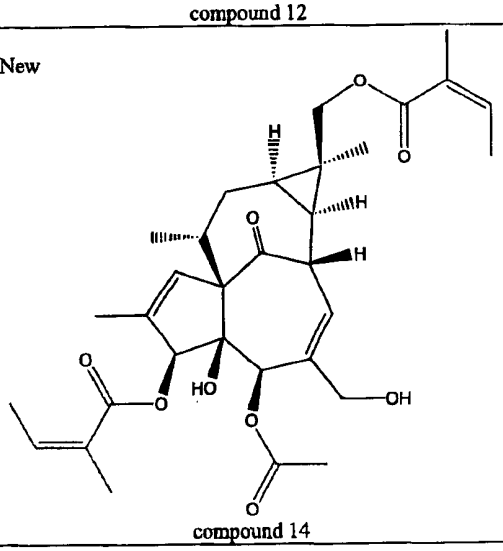
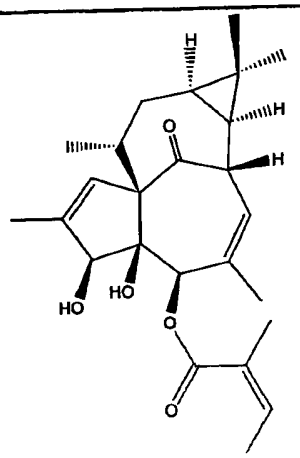
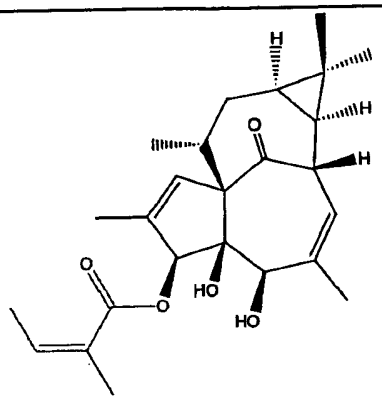
New  compound 9	New  compound 10
New  compound 11	New  compound 12
New  compound 13	New  compound 14

Table 6: Structures of Compounds 1-16 (c/fwd)

Known  compound 15	Known  compound 16

5. It is my considered scientific opinion that these data support the claim that cancer can be treated by administering to the subject in need thereof a therapeutically effective amount of an angeloyl-substituted ingenane obtainable from the sap of a *Euphorbia* species and an active derivative of an angeloyl-substituted ingenane obtainable from the sap of a *Euphorbia* species.

The undersigned declares further that all statements made herein are of his own knowledge, are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Date: August 22 2003

Dr. James Harrison Aylward

EXHIBIT JHA-1

CURRICULUM VITAE

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Present Work Location:

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Date of Birth: July 1, 1948, Springvale, Victoria, Australia

Present Position:

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Marital Status: Married, no children

Formal Education:

1972-75	PhD (Biochemistry) Monash University, Clayton, Victoria, Australia
1970-71	MSc qualifying (Biochemistry), Monash University, Clayton, Victoria, Australia
1967-69:	BSc, majors in Chemistry & Biochemistry, Monash University, Clayton, Victoria, Australia
1966:	Matriculation, Huntingdale High School, Huntingdale, Victoria, Australia

Professional Experience:

April 1998 – present time

Research Director, Peplin Biotech
direction of research relating to commercialisation of novel small molecules with biological activity, with focus on anticancer activity. Co-founder of Peplin Biotech in 1998

1992 - April 1998:

Principal Research Scientist CSIRO
Division of Tropical Agriculture
306 Carmody Road, St. Lucia, QLD 4068, Australia

Project Leader 1993-95 (Biotechnology group)
Budget responsibility: AUD $1.5m pa

improving the nutrition of ruminants by increasing the nutritive value of dietary fibre by manipulation of enzymes of fibre degradation in the rumen, using the tools of protein biochemistry and molecular biology

enzymes for use in the paper pulp industry

use of bacteria and yeasts as biocontrol agents for protection of fruits and vegetables from fungal spoilage

agents for use in opportunistic fungal infections and as immune system boosters

anti-cancer compounds which promote cellular differentiation

development of new functional foods

DNA incorporation into bacteria using sub micron gold particles

1984-91

Senior Research Scientist/Principal Research Scientist CSIRO Division of Tropical Animal Production, Meiers Road, Indooroopilly, QLD, 4068, Australia

vaccines against tick-borne diseases

1981-83

Research Scientist/Senior Research Scientist
CSIRO Division of Tropical Crops and Pastures, Cunningham Laboratory, St, Lucia QLD, 4068, Australia

nutritive value and toxicity testing of new dietary legumes (beans) for ruminants and monogastrics

1980-81 *Senior Tutor*
Monash University, Department of Biochemistry, Clayton, VIC, 3168, Australia

control of intermediary metabolism by fragments of growth hormone in muscle, adipose tissue and liver

1979-80 *Research Associate*
Department of Physiology
Howard Hughes Medical Institute
Vanderbilt University, Nashville, Tennessee, USA

mechanism of insulin and adrenalin action on muscle glycogen synthase, a key enzyme in control of carbohydrate metabolism

1976-78 *Research Associate*
Department of Biochemistry
University of Miami School of Medicine
Miami, Florida, USA

enzymology of phosphorylase phosphatase, a key enzyme in energy metabolism under hormonal control

Publications

Patent applications (CSIRO owned)

Inventors: **Aylward, J.H.** and Stone. B.F. (1991) "Tick paralysis toxin" *Australia 86784*

Inventors: Aylward, J.H. and Orpin, C.G. (1992) "Biocontrol bacteria" *Australia PL 0256*

Inventors: Williamson, M.A. and **Aylward, J.H.** (1992) "Biocontrol agents for use in horticulture" *Australia PL 8298*

Inventors: **Aylward, J.H.,** Riddles, P.W., and Wright, I.G. (1993) "Antigens and polypeptides derived from Babesia (12D3) antigen." *Australia 640398*

Inventors: **Aylward, J.H.** and Williamson, M.A. (1993) "Biocontrol agents for use in agricultural products" *Australia PL 7721*

Inventors: Xue, G-P., Gobius, K.S., **Aylward, J.H.**, and Orpin, C.G. (1993) "Recombinant cellulases"

Inventors: **Aylward, J.H.**, and Williamson, M.A. (1996) "Biocontrol agents in treatment of opportunistic infections" *Australia PN 9072*

Non-CSIRO owned

Inventor: **Aylward, J.H.** (1997) "Anti-cancer compounds" *Australia Provisional PO 8640,* PCT/AU98/00656 (transferred to Peplin Biotech Pty Ltd)

Papers and Book chapters

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Lee, E.Y.C., **Aylward, J.H.**, Mellgren, R.L., and Killilea, S.D. (1979) Protein phosphatase C: properties, specificity and structural relationship to a larger holoenzyme. In: *"From gene to protein: information transfer in normal and abnormal cells"* (Eds. T.R. Russell et al), pp. 483-500 (Academic Press, new York).

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Xue, G-P., Johnson, J.S., Dierens, L.M., Simpson, G.D., Denman, S.E., Goblus, K.S. and **Aylward, J.H.** (1994) Construction and purification of a recombinant fungal cellulase tagged with a flag peptide. In: *Proceedings of the Australian Society for Biochemistry and Molecular Biology* **26** POS-2-43. (The Society: South Melbourne).

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